

**Antioxidant properties and heat damage of water biscuits enriched with sprouted
wheat and barley**

Alyssa HIDALGO^a, Vesna TUMBAS ŠAPONJAC^b, Gordana ĆETKOVIĆ^b, Vanja ŠEREGELJ^b,
Jasna ČANADANOVIĆ-BRUNET^b, Doina CHIOSA^a, Andrea BRANDOLINI^{c*}

^aDipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS), Università degli
Studi di Milano, via G. Celoria 2, 20133 Milan, Italy.

^bFaculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21101 Novi Sad, Serbia.

^cConsiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), via Forlani 3,
26866 S. Angelo Lodigiano (LO), Italy

* Corresponding author e-mail: andrea.brandolini@crea.gov.it

14 **Abstract**

15 The germination process, fostered by renewed metabolic activities, leads to changes in many
16 characteristics of cereal kernels that improve their nutritional value. Aim of this study was to evaluate
17 protein, ash, sugars, heat damage (furosine, hydroxymethylfurfural, glucosylisomaltol), carotenoids,
18 tocols, phenols and antioxidant activity (FRAP, ABTS, DPPH, reducing power, superoxide anion, β -
19 carotene bleaching tests) of water biscuits enriched (0, 5, 10 and 20%) with sprouted meals of bread
20 wheat or barley. The meals from sprouted wheat and barley showed high concentrations of total
21 carotenoids (82.6 and 119.7 mg/kg, respectively), tocots (53.4 and 88.2 mg/kg), conjugated (368.0
22 and 564.2 mg/kg) and bound (1811.6 and 3022.0 mg/kg) phenols. The enriched water biscuits had
23 higher carotenoids, tocots and phenols content, heat damage and antioxidant activity than the
24 controls. The greatest increases were recorded for barley-enriched samples. Therefore, the addition of
25 15%-20% sprouted wheat or 5%-10% sprouted barley allowed to improve the nutritional quality of
26 water biscuits while limiting the heat damage.

27
28 **Key words.** Antioxidant activity; carotenoids; furosine; phenols; tocots.

29 **1. Introduction**

30 Cereals are the staple food of humankind because their edible kernels are rich sources of
31 carbohydrates, proteins, lipids, vitamins and minerals. During germination the activation of dormant
32 enzymes leads to significant changes in biochemical, nutritional and sensory characteristics. The α -
33 and β -amylases rapidly degrade the carbohydrates, with a consequent surge in reducing sugars, while
34 other enzymes degrade cell walls and enhance the availability of internal nutrients (Olaerts &
35 Courtin, 2018). The proteins are hydrolysed, increasing peptides and amino acids availability (Olaerts
36 & Courtin, 2018). Different vitamins, such as tocopherols, thiamine, riboflavin, folic acid and vitamin C,
37 as well as carotenoids, are biosynthesized to produce the nutrients for seedling growth (Olaerts &
38 Courtin, 2018). The metabolic activities fuel polyphenols biosynthesis (Benincasa et al., 2015), while
39 the degradation of cell walls leads to an increase of readily-available free phenolic acids (Van Hung,
40 Hatcher, & Barker, 2011). The more abundant presence of antioxidants molecules boosts the
41 antioxidant activity (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010). Sprouting increases
42 phytase activity, leading to phytic acid degradation and raise micronutrients absorption in the
43 gastrointestinal tract (Gupta, Gangoliya, & Singh, 2015; Olaerts & Courtin, 2018).

44 Controlled germination improves the nutritional composition of the kernels, but the hydrolytic
45 enzymes activity has a negative impact on some technological properties of cereals (e.g. bread wheat
46 leavening), indicating that the process must be carefully controlled to avoid excessive enzymatic
47 hydrolysis (Olaerts & Courtin, 2018). On non-leavened products, the effects of germination on
48 technological characteristics are less important. In pasta making, sprouting decreases endosperm
49 vitreousness and falling number of durum wheat, but spaghetti quality for colour, breakage, cooking
50 losses and consistency was comparable to controls prepared from non-sprouted kernels (Dick, Walsh,
51 & Gilles, 1974). More recently, Fu, Hatcher and Schlichting (2014) studied pasta with up to 50%
52 sprouted durum wheat semolina and found that proteins and colour were unaffected, vitreousness
53 decreased and α -amylase activity increased but gluten index and alveogram parameters showed only
54 minimal changes. After drying at 85 °C, browning was observed exclusively in samples with more

55 than 35% sprouted semolina, and after cooking differences in rheological properties (less consistency
56 and higher dry matter loss) were perceived only in 100% sprouted semolina spaghetti. In cookies,
57 only minor changes are reported, i.e. an increase in spread and an improvement in top grain along
58 with a darker colour of the crust (An, 2015; Lorenz & Valdano, 1981).

59 The growing attention of the consumers for foods that not only satisfy the nutritional needs of the
60 organism, but which also aid in the prevention of diseases is fostering the study of new formulations
61 and the marketing of new products. In this context, the utilisation of sprouted cereal kernels in
62 snacks, pasta and bakery products is gaining momentum, because sprouted grains are perceived as
63 healthful ingredients. Therefore, the purpose of this study was to evaluate some nutritional
64 characteristics, viz. protein, ash, sugars, furosine, carotenoids, tocols and phenolic compounds
65 content, and antioxidant activity, of bread wheat water biscuits prepared with the addition of
66 increasing concentrations (0, 5, 10 and 20%) of meals obtained from sprouted wheat or barley
67 kernels.

68

69 **2. Materials and methods**

70

71 *2.1. Materials*

72 The biscuit-quality bread wheat cv. Bramante was cropped in 2016-17 in the Sant'Angelo Lodigiano
73 fields of CREA. After harvest, the seeds were milled with a lab mill (Bona, Monza, Italy) that
74 separated the flour from the bran and the germ; the flour was stored at -20 °C until analysis and water
75 biscuit manufacturing. The sprouted kernel meals were prepared as detailed by Aborus et al. (2018)
76 from bread wheat cv. 'Simonida' and barley hybrid 'NS565', kindly provided by the Institute of Field
77 and Vegetable Crops (NS seed), Novi Sad, Republic of Serbia; the meals were packaged in plastic
78 bags and stored at -20 °C until analysis and water biscuit manufacturing.

79

80 *2.2. Water biscuits preparation*

81 The water biscuits were made only with deionized water and flour or with deionized water, freeze-
82 dried sprouted meal (either wheat or barley) and flour, to avoid the interference of other ingredients
83 used in biscuits and cookies (i.e. fat, sugar and skimmed powdered milk). The control water biscuits
84 were prepared from 80 g bread wheat flour, adjusted to 13.5% humidity, and 40 mL deionized water,
85 while in the sprout-enriched water biscuits 5%, 10% and 20% flour was replaced by equal amounts of
86 barley or wheat sprout meal. The deionized water used for each dough was adjusted considering the
87 moisture of the ingredients. Every dough was kneaded for 90 s with a Hobart C-100 electric mixer
88 (National MFG CO, Lincoln, Nebraska, U.S.A.), rolled to a uniform 3.9 mm thinness and cut with a
89 die-cutter (internal diameter 35 mm), obtaining 12 water biscuits. The water biscuits were cooked in
90 a rotating oven (Ovenlab, National MFG CO, Lincoln, Nebraska, U.S.) at 205 °C for 18 min, cooled
91 at room temperature for 30 min and stored at -20 °C under vacuum in plastic bags. Two different sets
92 of water biscuits were prepared. The water biscuits were ground with a laboratory mill (Braun,
93 Germany) just before the analysis.

94

95 *2.3. Methods*

96 Water activity was assessed at 25 °C using an AquaLab laboratory tool (Decagon Devices Inc.,
97 Washington, USA). Dry matter (DM) was determined gravimetrically, according to method 44-15
98 (AACC, 1995); protein content was determined by method 46-10 (AACC, 1995), employing 5.70 as
99 multiplying factor; ash content was scored according to method 08-03 (AACC, 1995); fructose,
100 glucose, maltose and sucrose were assessed by HPLC, following Hidalgo and Brandolini (2011); all
101 these results are reported as g/100 g DM. Furosine was determined by HPLC as described by Hidalgo
102 and Brandolini (2011) and the results are expressed as milligrams furosine/100 g protein.
103 Hydroxymethylfurfural (HMF) and glucosylisomaltol (GLI) were determined following the HPLC
104 method of Rufián-Henares, Delgado-Andrade and Morales (2008) as described by Hidalgo and
105 Brandolini (2011); the results are expressed as mg/kg DM. Carotenoids and tocopherols were extracted and
106 quantified by NP-HPLC as described by Hidalgo, Brandolini and Pompei (2010), and by Hidalgo and

Brandolini (2010), respectively; the soluble conjugated and the insoluble bound phenols of flour, sprouted meals and water biscuits were extracted and analysed as described by Yilmaz, Brandolini and Hidalgo (2015); the results are reported as mg/kg DM.

The antioxidant activity was assessed following six different methods: ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), as described by Yilmaz et al. (2015); reducing power according to Oyaizu (1986); superoxide anion following Gironés-Vilaplana et al. (2012), and β -carotene bleaching (BCB) as outlined by Al-Saikhan, Howard and Miller (1995). The tests were performed on 70% methanol extracts, except for the β -carotene bleaching test which was done both on 70% methanol and on hexane extracts. The antioxidant activity was expressed as millimoles of Trolox equivalent (TE) per kilogram DM. All the analyses were performed in duplicate on flour, sprouted meals and water biscuits; different water biscuits were tested.

2.4. Statistical analysis

The data were processed by analysis of variance (ANOVA), considering as factors the different water biscuit types. When significant differences were found ($p \leq 0.05$), Fisher's lowest significant difference (LSD) at 95% significance level was computed. The analyses were performed using the Statgraphics® Centurion XVI statistical program (Statpoint Technologies, Inc., USA). The average values and the standard deviation were calculated using the Excel program (Microsoft® Office Excel 2016).

3. Results and discussion

3.1. Flour and meals

The ANOVA (not presented) performed on the characteristics of the flour and of the sprouted meals highlighted significant differences for all the parameters studied. Table 1 reports the average values

132 of protein, ash, sugars and furosine for the three types of raw materials (bread wheat flour, sprouted
 133 wheat meal and sprouted barley meal) used in water biscuit production.

134 The characteristics of Bramante flour are in line with the information available in literature (Faridi,
 135 Gaines, & Stout, 2000; Hidalgo & Brandolini, 2011; Hidalgo, Brandolini, Čanadanović-Brunet,
 136 Ćetković, & Tumbas-Šaponjac, 2018). The sprouted meals had protein and ash content higher than
 137 the white flour, but similar to non-sprouted kernels, i.e. 12.9-19.9 g/100 g and 1.49-2.05 g/100 g in
 138 wheat (Rakšzegi et al., 2008), and 8.1-14.7 g/100 g and 1.8-3.0 g/100 g in barley (Andersson et al.,
 139 2008; Jadhav, Lutz, Ghorpade, & Salunkhe, 1998).

140 The sugars concentration in the flour was very low, as observed also by Hidalgo et al. (2018) and
 141 Hidalgo and Brandolini (2011); glucose was the most abundant reducing sugar, followed by maltose,
 142 while the non-reducing sucrose was highest in bread wheat meal. The sprouted meals had higher
 143 concentrations of all sugars. Glucose was more abundant than in the sprouted wheats and barleys
 144 (0.21-0.43 and 0.15-0.53 g/100 g DM, respectively) analysed by Panfil et al. (2014), probably
 145 because of different sprouting conditions. Our kernels were exposed to light during their germination,
 146 and Ziegler et al. (2016) demonstrated that the α -amylase activity, which degrades the starch to
 147 simple fermentable sugars, is strongly increased in wheat seeds germinated under illumination.

148 The high furosine values observed in sprouted meals (68.1 and 109.8 mg/100 g protein for wheat and
 149 barley) are due to the presence of more reducing sugars and amino acids after germination (Lorenz &
 150 D'Appolonia, 1980; Ziegler et al., 2016), that stimulate the Maillard reaction and the development of
 151 related molecules.

152 The main antioxidant compounds (carotenoids, tocopherols and polyphenols) and the antioxidant activity
 153 of flour and sprouted meals are presented in Table 2. The bread wheat flour had low carotenoid
 154 concentration, in line with literature reports (Hidalgo & Brandolini, 2008; Ziegler et al., 2016), and a
 155 total tocopherols content comparable to the data reported by Hidalgo and Brandolini (2008). The
 156 carotenoid content was very high in the sprouted meals of wheat and, even more, of barley. Hence,
 157 the carotenoid concentrations of the sprouted meals were in the same range of highly-touted

158 vegetables such as carrot (283 mg/kg fresh weight; Surles, Weng, Simon, & Tanumihardjo, 2004)
 159 and spinach (176.6-226.3 mg/kg fresh weight; Kidmose, Knuthsen, Edelenbos, Justesen, &
 160 Hegelund, 2001). Lutein was always the predominant pigment, but in both meals ($\alpha+\beta$)-carotene
 161 content largely exceeded that of the non-sprouted flour. Ziegler et al. (2016) described a limited
 162 carotenoid increase (from 1.3 to 2.0 mg/kg DM) in dark-sprouted wheat but observed that
 163 illumination favoured carotenoid synthesis (from 1.3 to 9.3 mg/kg DM) along with chlorophyll
 164 formation. Light induces the biogenesis of chloroplasts, which accumulate carotenoids as accessory
 165 pigments or photoprotective constituents of their photosynthetic protein complexes (Sun et al., 2018).
 166 The tocols content of sprouted bread wheat meal was within the variation (53.2-74.9 mg/kg DM)
 167 described by Hidalgo, Brandolini, Pompei and Piscozzi (2006) for non-sprouted seeds; the
 168 germination spurred the synthesis of γ -tocopherol (10.1 mg/kg DM), while the other homologues
 169 were in the range observed by Hidalgo et al. (2006). The tocols content of sprouted barley meal was
 170 higher than those (46.2-68.8 and 28.4-41.6 mg/kg DM) reported by Andersson et al. (2008) and
 171 Lachman, Hejtmánková, Orsák, Popov and Martinek (2018), respectively, for non-sprouted barley
 172 kernels.

173 In wheat three types of phenols are present: soluble free, insoluble conjugated (esterified to sugars
 174 and other low molecular weight components) and insoluble bound (linked to cell wall constituents
 175 such as polysaccharides, protein, lignin, cutin or suberin). In our analysis we did not survey the free
 176 phenols. Aborus et al. (2017) and Aborus et al. (2018) found free phenols in sprouted wheat and
 177 barley kernels, but other authors (e.g. Alvarez-Jubete et al., 2010) did not record their presence,
 178 maybe because they are scarce, very unstable, rapidly degradable and therefore rarely detectable. The
 179 conjugated and bound polyphenols in sprouted wheat meal (Table 2) were significantly more
 180 abundant than those of non-sprouted whole bread wheat meal, i.e. 40.2-46.9 and 612-647 mg/kg DM
 181 (Brandolini, Castoldi, Plizzari, & Hidalgo, 2013), implying that these compounds are synthesized
 182 during germination. An increase in polyphenols content after sprouting was observed by Žilić et al.
 183 (2014) and, limited to the free form, by Benincasa et al. (2015). Ferulic acid was the most abundant

phenol in both fractions, followed by gentisic acid (conjugated phenols) and salicylic acid (bound phenols). The conjugated and bound phenols in sprouted barley meal were more abundant than in sprouted wheat, as well as above the range reported by Idehen, Tang and Sang (2017) for non-sprouted barley (86-198 DM and 133-523 mg/kg DM) and by Dvořáková et al. (2008) for sprouted barley (46.7-70.2 and 129.1-305.7 mg/kg). Ferulic acid was again the most abundant phenol, at concentrations higher than those described by Andersson et al. (2008) (21.7-42.5 and 104.3-365.4 mg/kg DM) and Dvořáková et al. (2008) (33.3-53.5 and 113.1-242.5 mg/kg DM). A flavonoid, kaempferol, was also present in both sprouted meals. Hence, our results demonstrate that during germination some phenolic compounds are synthesized thanks to the activation of specific metabolic pathways.

The *in vitro* antioxidant activity is better determined with different tests; thus, to understand the effects of the different hydrophilic (polyphenols and flavonoids) and lipophilic (carotenoids and tocopherols) compounds in a more comprehensive way, the antioxidant activity was challenged by several methods. All the tests showed that the activity of the sprouted meals was significantly higher than that of the flour (Table 2). The reducing power assay had slightly lower values than FRAP, the other method based on the ferric ion reduction mechanism.

FRAP, ABTS, DPPH and reducing power tests indicated that bread wheat flour had limited antioxidant activity, probably because phenols are scarce in refined flour and polar extraction solutions (e.g. methanol:water 70:30) do not recover adequately the lipophilic antioxidants; the superoxide anion and BCB assays showed trends similar to the other tests.

3.2. Water biscuits

The ANOVA (not presented) showed that the percentage of enrichment was the main factor influencing furosine content, while both percentage of enrichment and flour/meal type modified HMF, GLI, carotenoids, tocopherols, conjugated and bound phenols, and antioxidant activity. The interactions, often significant, had a more limited influence.

210 Fig. 1 depicts the heat damage in the different water biscuits. Some furosine (93.0 mg/100g protein)
 211 was present in the control sample despite the low sugar content of the wheat flour, while other
 212 intermediate products of the Maillard reaction were absent; a similar result (110 mg furosine/100 g
 213 proteins; no HMF and GLI) was reported by Hidalgo et al. (2018) in bread wheat water biscuits.
 214 Furosine, HMF and GLI contents increased with augmenting percentages of sprouted meals. The
 215 samples enriched with sprouted wheat in general showed a lower thermal damage (furosine: 416.1
 216 mg/100 g proteins; HMF: 1.4 mg/kg DM; GLI: 0.8 mg/kg DM) compared to those enriched with
 217 sprouted barley (furosine: 473.1 mg/100 g protein; HMF: 3.3; GLI: 1.3 mg/kg DM).

218 Fig. 2 depicts the total carotenoids, tocopherols, conjugated phenols and bound phenols content of the
 219 control and of the sprouted meal enriched water biscuits; the detailed phenols composition is reported
 220 in Supplementary Table 1. All the compounds increased with the sprouted meals enrichment: a 20%
 221 addition raised carotenoid content by 666.7% in the samples with sprouted wheat and by 1666.7% in
 222 those with sprouted barley. The ($\alpha+\beta$)-carotene had the greatest proportional increase (on average
 223 from 0.1 to 5.3 mg/kg DM), followed by lutein (from 1.0 to 9.2 mg/kg DM). The water biscuits with
 224 sprouted barley had about twice the carotenoids contents than those with sprouted wheat; the only
 225 exception was zeaxanthin, with similar concentrations in both species. The increase in tocopherols content
 226 was limited (22.6%) in those with sprouted wheat and strong (93.5%) in those with sprouted barley.
 227 Furthermore, in the latter a new compound (γ -tocotrienol), absent in flour and sprouted barley meal,
 228 was formed. The homologues with the greatest increase were α -tocopherol and α -tocotrienol,
 229 particularly in water biscuits with sprouted barley. The phenolic compounds increased too: at 20%
 230 addition they reached 111.5 and 161.1 mg/kg DM (conjugated compounds in wheat and barley,
 231 respectively) as well as 533.9 and 1115.0 mg/kg DM (bound compounds in wheat and barley,
 232 respectively). Gentisic and ferulic acids showed the greatest raise among conjugated phenols, ferulic
 233 acid and salicylic acid among bound phenols. The flavonoid kaempferol, both in the conjugate and
 234 the bound form, increased proportionally to the percentage of enrichment. In general, the samples

235 with sprouted barley meal showed the highest increase in each phenol, except syringic acid;
 236 chlorogenic acid was detected only in barley. Overall, therefore, the water biscuits enriched with
 237 sprouted barley had the biggest quantities of carotenoids, tocopherols and phenolic compounds. An
 238 increase of most free phenolic acids in breads enriched with different quantities of sprouted wheat
 239 was recorded also by Gawli-Dziki, Dziki, Pietrzak and Nowak (2017).

240 A consequence of the carotenoids, tocopherols and polyphenols increase in water biscuits enriched with
 241 sprouted meals was a strong growth in antioxidant activity (Fig. 3). From very low in the control
 242 (FRAP: 0.7; ABTS: 1.2; DPPH: not detected; reducing power: 0.4 mmol TE/kg DM), high values
 243 were reached both in the sprouted wheat enriched water biscuits (with 20% meal: 3.6, 2.8, 0.9 and 3.4
 244 mmol TE/kg DM, respectively) and in those with sprouted barley (with 20% meal: 9.9, 6.1, 1.8 and
 245 5.8 mmol TE/kg DM). Similarly, the superoxide anion and β -carotene bleaching methanol and
 246 hexane tests showed an increase in antioxidant activity: from 32.9, 12.2 and 40.4 mmol TE/kg DM in
 247 the control to 48.8, 12.4 and 74.3 mmol TE/kg DM for wheat sprouted-meal enriched and to 62.3, 18.4
 248 and 96.7 mmol TE/kg DM for barley sprouted-meal enriched water biscuits. The samples
 249 supplemented with sprouted barley almost always exhibited an antioxidant activity superior to the
 250 sprouted wheat enriched water biscuits.

251 During water biscuits manufacturing some loss of antioxidant molecules was observed. The total
 252 carotenoids and tocopherols content of the control was slightly lower (1.2 and 15.4 mg/kg DM) than that of
 253 the flour (1.8 and 24.3 mg/kg DM); in contrast, some phenolic compounds (conjugated vanillic acid,
 254 ferulic acid and bound salicylic acid) increased, probably because the production steps destabilised
 255 their links with some molecules, e.g. arabinose and short arabinoxylan chains (conjugated
 256 compounds), cellulose and lignin (bound compounds) (Dvořáková et al., 2008). For the enriched
 257 water biscuits, a theoretical degradation was computed considering flour/meals composition and
 258 percentage addition. Thus, total carotenoids loss in sprouted wheat enriched samples varied from
 259 39% to 49%, and in sprouted barley enriched samples from 16% to 27%; interestingly, β -
 260 cryptoxanthin increased during baking, confirming the report of Hidalgo et al. (2010). For tocopherols, the

261 theoretical loss in sprouted wheat enriched water biscuits was slightly lower than for carotenoids (35-
262 37%), while in sprouted barley enriched samples the loss varies from 19 to 42%. On the contrary, the
263 total phenolic content increased in both sprouted wheat and sprouted barley enriched water biscuits
264 (16-43% and 61-82%, respectively).

265

266 **4. Conclusions**

267 The germination favoured the synthesis of carotenoids (particularly lutein and ($\alpha+\beta$)-carotene),
268 conjugated and bound phenols and antioxidant activity. In water biscuits, furosine, HMF and GLI
269 grew according to the enrichment percentages, but more with sprouted barley addition. Carotenoids,
270 conjugated and bound phenolic compounds and antioxidant activity were higher in the enriched
271 samples than in the control; this increase was not observed for tocopherols, except with sprouted barley
272 flour. Water biscuits preparation and baking led to some degradation of antioxidant compounds.
273 In conclusion, the addition of wheat or barley sprouted seeds meal improves the nutritional quality of
274 bakery products. A 15-20% (sprouted wheat) or 5-10% (sprouted barley) enrichment improves
275 carotenoid, tocopherol and polyphenol content while maintaining thermal damage low.

276

277 **Acknowledgements**

278 The Authors thank Provincial Secretariat for Science and Technological Development, autonomous
279 province of Vojvodina, Republic of Serbia, for financial support (Grant n° 114-451-2467/2018-
280 01/02).

281 **References**

- 282 AACC (American Association of Cereal Chemists). (1995). AACC Official Methods 08-03, 44–15,
283 46-10. In: Approved Methods of the American Association of Cereal Chemists. AACC,
284 Minneapolis, USA.
- 285 Aborus N. E., Tumbas Šaponjac, V., Čanadanović-Brunet, J., Četković, G., Hidalgo, A., Vulić, J., &
286 Seregelli, V. (2018). Sprouted and freeze-dried wheat and oat seeds - phytochemical profile and *in*
287 *vitro* biological activities. *Chemistry & Biodiversity*, 15, e1800119.
- 288 Aborus, N. E., Čanadanović-Brunet, J., Četković, G., Tumbas Šaponjac, V., Vulić, J., & Ilić, N.
289 (2017). Powdered barley sprouts: composition, functionality and polyphenol digestibility.
290 *International Journal of Food Science & Technology*, 52, 231-238.
- 291 Al-Saikhan, M. S., Howard, L. R., & Miller Jr, J. C. (1995). Antioxidant activity and total phenolics
292 in different genotypes of potato. *Journal of Food Science*, 60, 341-343.
- 293 Alvarez-Jubete, L., Wijngaard, H., Arendt, E. K., & Gallagher, E. (2010). Polyphenol composition
294 and *in vitro* antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by
295 sprouting and baking. *Food Chemistry*, 119, 770-778.
- 296 An, S. H. (2015). Quality characteristics of cookies made with added wheat sprout powder. *Korean*
297 *Journal of Food and Cookery Science*, 31, 687-695.
- 298 Andersson, A. A., Lampi, A. M., Nyström, L., Piironen, V., Li, L., Ward, J. L., ...& Fras, A. (2008).
299 Phytochemical and dietary fiber components in barley varieties in the HEALTHGRAIN diversity
300 screen. *Journal of Agricultural and Food Chemistry*, 56, 9767-9776.
- 301 Benincasa P., Galieni A., Manetta A. C., Pace R., Guiducci M., Pisante M., & Stagnari F. (2015).
302 Phenolic compounds in grains, sprouts and wheatgrass of hulled and non-hulled wheat species.
303 *Journal of the Science of Food and Agriculture*, 95, 1795-1803.
- 304 Brandolini, A., Castoldi, P., Plizzari, L., & Hidalgo, A. (2013). Phenolic acids composition, total
305 polyphenols content and antioxidant activity of *Triticum monoccocum*, *Triticum turgidum* and
306 *Triticum aestivum*: a two-years evaluation. *Journal of Cereal Science*, 58, 123-131.

307 Dick J. W., Walsh D. E., & Gilles K. A. (1974). The effect of field sprouting on the quality of durum
 308 wheat. *Cereal Chemistry*, 51, 180-187.

309 Dvořáková, M., Guido, L. F., Dostálek, P., Skulilová, Z., Moreira, M. M., & Barros, A. A. (2008).
 310 Antioxidant properties of free, soluble ester and insoluble-bound phenolic compounds in different
 311 barley varieties and corresponding malts. *Journal of the Institute of Brewing*, 114, 27-33.

312 Faridi, H., Gaines, C. S., & Strout, B. L. (2000). Soft wheat products. In K. Kulp (Ed.) *Handbook of*
 313 *cereal science and technology* (pp. 575-614). CRC Press.

314 Fu B. X., Hatcher D. W., & Schlichting L. (2014). Effects of sprout damage on durum wheat milling
 315 and pasta processing quality. *Canadian Journal of Plant Science*, 94, 545-553.

316 Gawlik-Dziki, U., Dziki, D., Pietrzak, W., & Nowak, R. (2017). Phenolic acids prolife and
 317 antioxidant properties of bread enriched with sprouted wheat flour. *Journal of Food Biochemistry*,
 318 41, e12386.

319 Gironés-Vilaplana, A., Valentão, P., Andrade, P. B., Ferreres, F., Moreno, D. A., & García-Viguera,
 320 C. (2012). Phytochemical profile of a blend of black chokeberry and lemon juice with
 321 cholinesterase inhibitory effect and antioxidant potential. *Food Chemistry*, 134, 2090–2096.

322 Gupta, R. K., Gangoliya, S. S., & Singh, N. K. (2015). Reduction of phytic acid and enhancement of
 323 bioavailable micronutrients in food grains. *Journal of Food Science and Technology*, 52, 676-684.

324 Hidalgo A., & Brandolini A. (2008). Protein, ash, lutein and tocols distribution in einkorn (*Triticum*
 325 *monococcum* L. subsp. *monococcum*) seed fractions. *Food Chemistry*, 107, 444-448.

326 Hidalgo A., Brandolini A., Pompei C., & Piscozzi R. (2006). Carotenoids and tocols of einkorn
 327 wheat (*Triticum monococcum* ssp. *monococcum* L.). *Journal of Cereal Science*, 44, 182-193.

328 Hidalgo, A., & Brandolini, A. (2010). Tocols stability during bread, water biscuit and pasta
 329 processing from wheat flours. *Journal of Cereal Science*, 52, 254-259.

330 Hidalgo, A., & Brandolini, A. (2011). Heat damage of water biscuits from einkorn, durum and bread
 331 wheat flours. *Food Chemistry*, 128, 471-478.

332 Hidalgo, A., Brandolini, A., & Pompei, C. (2010). Carotenoids evolution during pasta, bread and
 333 water biscuit preparation from wheat flours. *Food Chemistry*, 121, 746–751.

334 Hidalgo, A., Brandolini, A., Čanadanović-Brunet J., Četković, G., & Tumbas-Šaponjac, V. (2018).
 335 Microencapsulates and extracts from red beetroot pomace modify antioxidant capacity, heat
 336 damage and colour of pseudocereals-enriched einkorn water biscuits. *Food Chemistry*, 268, 40-48.

337 Idehen, E., Tang, Y., & Sang, S. (2017). Bioactive phytochemicals in barley. *Journal of Food and*
 338 *Drug Analysis*, 25, 148-161.

339 Jadhav, S. J., Lutz, S. E., Ghorpade, V. M. & Salunkhe D. K. (1998). Barley: chemistry and value-
 340 added processing. *Critical Reviews in Food Science and Nutrition*, 38, 123-171.

341 Kidmose, U., Knuthsen, P., Edelenbos, M., Justesen, U., & Hegelund, E. (2001). Carotenoids and
 342 flavonoids in organically grown spinach (*Spinacia oleracea* L.) genotypes after deep frozen
 343 storage. *Journal of the Science of Food and Agriculture*, 81, 918-923.

344 Lachman, J., Hejtmánková, A., Orsák, M., Popov, M., & Martinek, P. (2018). Tocotrienols and
 345 tocopherols in colored-grain wheat, tritordeum and barley. *Food Chemistry*, 240, 725-735.

346 Lorenz, K., & D'Appolonia, B. (1980). Cereal sprouts: composition, nutritive value, food
 347 applications. *Critical Reviews in Food Science and Nutrition*, 13, 353-385.

348 Lorenz, K., & Valvano, R. (1981). Functional characteristics of sprout-damaged soft white wheat
 349 flours. *Journal of Food Science*, 46, 1018-1020.

350 Olaerts, H., & Courtin, C. M. (2018). Impact of preharvest sprouting on endogenous hydrolases and
 351 technological quality of wheat and bread: a review. *Comprehensive Reviews in Food Science and*
 352 *Food Safety*, 17, 698-713.

353 Oyaizu, M. (1986). Studies on products of browning reaction—antioxidant activities of products of
 354 browning reaction prepared from glucoamine. *Japanese Journal of Nutrition*, 44, 307–315.

355 Panfil, P., Dorica, B., Sorin, C., Emilian, M., Ersilia, A., & Iosif, G. (2014). Biochemical
 356 characterization of flour obtained from germinated cereals (wheat, barley and oat). *Romanian*
 357 *Biotechnological Letters*, 19, 9772-9777.

358 Rakszegi, M., Boros, D., Kuti, C., Láng, L., Bedo, Z., & Shewry, P. R. (2008). Composition and end-
 359 use quality of 150 wheat lines selected for the HEALTHGRAIN diversity screen. *Journal of*
 360 *Agricultural and Food Chemistry*, 56, 9750–9757.

361 Rufián-Henares, J. A., Delgado-Andrade, C., & Morales, F. J. (2008). Relevance of
 362 glucosylisomaltol and galactosylisomaltol in commercial biscuits. *European Food Research and*
 363 *Technology*, 227, 1447.

364 Sun, T., Yuan, H., Cao, H., Yazdani, M., Tadmor, Y., & Li, L. (2018). Carotenoid metabolism in
 365 plants: the role of plastids. *Molecular Plant*, 11, 58-74.

366 Surles, R. L., Weng, N., Simon, P. W., & Tanumihardjo, S. A. (2004). Carotenoid profiles and
 367 consumer sensory evaluation of specialty carrots (*Daucus carota*, L.) of various colors. *Journal of*
 368 *Agricultural and Food Chemistry*, 52, 3417-3421.

369 Van Hung P., Hatcher D. W., & Barker W. (2011). Phenolic acid composition of sprouted wheats by
 370 ultra-performance liquid chromatography (UPLC) and their antioxidant activities. *Food*
 371 *Chemistry*, 126, 1896-1901.

372 Yilmaz, V. A., Brandolini, A., & Hidalgo, A. (2015). Phenolic acids and antioxidant activity of wild,
 373 feral and domesticated diploid wheats. *Journal of Cereal Science*, 64, 168-175.

374 Ziegler, J. U., Flockerzie, M., Longin, C. F. H., Würschum, T., Carle, R., & Schweiggert, R. M.
 375 (2016). Development of lipophilic antioxidants and chloroplasts during the sprouting of diverse
 376 *Triticum* spp. *Journal of Agricultural and Food Chemistry*, 64, 913-922.

377 Žilić, S., Basić, Z., Hadži-Tašković Šukalović, V., Maksimović, V., Janković, M., & Filipović, M.
 378 (2014). Can the sprouting process applied to wheat improve the contents of vitamins and phenolic
 379 compounds and antioxidant capacity of the flour? *International Journal of Food Science and*
 380 *Technology*, 49, 1040-1047.

381 **Legends to Figures**

382

383 **Figure 1.** Furosine, hydroxymethylfurfural (HMF) and glycosylisomaltol (GLI) content of biscuits
384 obtained from bread wheat flour, without (Control) and with the addition (5%, 10% and 20%) of
385 sprouted bread wheat meal or sprouted barley meal. Different letters indicate significant difference
386 between the samples according to the LSD test ($p \leq 0.05$).

387

388 **Figure 2.** Total carotenoids, tocopherols, conjugated phenolics and bound phenolics content of biscuits
389 obtained from bread wheat flour, without (Control) and with the addition (5%, 10% 20%) of
390 sprouted bread wheat meal or sprouted barley meal. Different letters indicate significant difference
391 between samples according to the LSD test ($p \leq 0.05$).

392

393 **Figure 3.** Antioxidant activity following FRAP, ABTS, DPPH, reducing power, superoxide anion,
394 and beta-carotene bleaching (BCB) tests of methanol extracts and BCB of hexane extracts of
395 biscuits obtained from bread wheat flour, without (Control) and with the addition (5%, 10% 20%)
396 of sprouted bread wheat meal or sprouted barley meal. Different letters indicate significant
397 difference between samples according to the LSD test ($p \leq 0.05$).

398

Table 1. Means (\pm standard deviation) and LSD test results ($p \leq 0.05$) for water activity (a_w), moisture content (g/100 g), protein, ash, sugars (g/100 g DM) and furosine (mg/100 g protein) in bread wheat flour, sprouted bread wheat meal (WS) and sprouted barley meal (BS). For each parameter, different letters indicate significant difference between the raw materials.

	Bread wheat flour	WS	BS
Protein	8.5 ^c \pm 0.0	10.3 ^b \pm 0.0	12.1 ^a \pm 0.0
Ash	0.5 ^c \pm 0.0	1.8 ^b \pm 0.1	3.1 ^a \pm 0.0
Fructose	0.0 ^c \pm 0.0	0.8 ^b \pm 0.0	1.7 ^a \pm 0.1
Glucose	0.1 ^c \pm 0.0	3.5 ^b \pm 0.2	7.0 ^a \pm 0.5
Maltose	0.2 ^b \pm 0.0	2.2 ^a \pm 0.2	2.5 ^a \pm 0.0
Sucrose	0.4 ^c \pm 0.0	2.9 ^a \pm 0.3	1.6 ^b \pm 0.1
Furosine	6.7 ^c \pm 0.3	68.1 ^b \pm 0.1	109.8 ^a \pm 3.9

406 **Table 2.** Means (\pm standard deviation) and LSD test results ($p \leq 0.05$) for content in carotenoids, tocols, soluble
 407 conjugated and insoluble bound phenolic compounds (mg/kg DM), and antioxidant activity following FRAP, ABTS,
 408 DPPH, reducing power, superoxide anion, beta-carotene bleaching (BCB) test of methanol extracts and BCB of hexane
 409 extract (mmoles TE/kg DM) of bread wheat flour, sprouted bread wheat meal (WS) and sprouted barley meal (BS).
 410 Different letters indicate significant difference between raw materials.
 411

	Bread wheat flour	WS	BS
<i>Carotenoids</i>			
(α + β)-carotene	0.1 ^b \pm 0.0	39.0 ^a \pm 3.9	44.9 ^a \pm 1.1
β -cryptoxanthin	nd	0.1 ^b \pm 0.0	0.2 ^a \pm 0.0
Lutein	1.6 ^c \pm 0.2	42.5 ^b \pm 0.4	73.1 ^a \pm 3.7
Zeaxanthin	0.1 ^b \pm 0.0	1.0 ^a \pm 0.0	1.5 ^a \pm 0.3
Total carotenoids	1.8^c \pm 0.2	82.6^b \pm 3.5	119.7^a \pm 2.8
<i>Tocols</i>			
α -tocopherol	3.5 ^c \pm 0.1	10.1 ^b \pm 0.9	13.5 ^a \pm 0.9
α -tocotrienol	0.5 ^b \pm 0.1	4.0 ^b \pm 0.7	31.6 ^a \pm 1.9
β -tocopherol	3.7 ^c \pm 0.8	7.1 ^b \pm 0.7	11.3 ^a \pm 0.6
β -tocotrienol	16.6 ^b \pm 2.5	22.0 ^a \pm 0.0	nd
γ -tocopherol	nd	10.1 ^b \pm 0.4	25.4 ^a \pm 2.9
γ -tocotrienol	nd	nd	6.4 ^a \pm 0.4
Total tocols	24.3^c \pm 3.6	53.4^b \pm 2.7	88.2^a \pm 4.9
<i>Conjugated phenolic acids</i>			
Chlorogenic	nr	nr	19.4 \pm 0.1
Gentisic	0.0 ^c \pm 0.0	110.6 ^b \pm 14.4	141.3 ^a \pm 1.4
Vanillic	3.8 ^b \pm 0.2	48.0 ^a \pm 1.5	47.3 ^a \pm 0.1
Syringic	0.9 ^c \pm 0.0	22.9 ^a \pm 2.3	6.5 ^b \pm 0.2
Coumaric	0.0 ^c \pm 0.0	42.0 ^a \pm 0.7	35.5 ^b \pm 2
Ferulic	4.2 ^c \pm 0.2	114.1 ^b \pm 2.0	187.3 ^a \pm 1.2
Salicylic	0.0 ^c \pm 0.0	25.4 ^b \pm 2.0	113.9 ^a \pm 3.7
Kaempferol	0.7 ^c \pm 0.1	5.1 ^b \pm 0.5	13.0 ^a \pm 1.3
Total conjugated	9.6^c \pm 0.1	368.0^b \pm 13.2	564.2^a \pm 5.9
<i>Bound phenolic acids</i>			
<i>p</i> -hydroxybenzoic	0.0 ^b \pm 0.0	9.1 ^a \pm 0.8	10.5 ^a \pm 1.1
Gentisic	0.0 ^c \pm 0.0	22.0 ^b \pm 0.7	40.4 ^a \pm 0.1
Vanillic	0.5 ^c \pm 0.1	9.0 ^b \pm 0.6	13.3 ^a \pm 0.9
Syringic	0.0 ^c \pm 0.0	5.3 ^a \pm 0.3	2.9 ^b \pm 0.2
Coumaric	2.2 ^c \pm 0.2	21.9 ^b \pm 1.9	67.0 ^a \pm 3.3
Ferulic	48.1 ^c \pm 2.2	1034.4 ^b \pm 79.7	1450.9 ^a \pm 137.8
Salicylic	23.3 ^c \pm 1.2	705.3 ^b \pm 50.9	1399.4 ^a \pm 89.2
Kaempferol	0.0 ^c \pm 0.0	4.6 ^b \pm 1.2	37.6 ^a \pm 2.3
Total bound	74.2^c \pm 3.5	1811.6^b \pm 130.1	3022.0^a \pm 234.8
<i>Antioxidant activity</i>			
FRAP	0.2 ^c \pm 0.0	10.9 ^b \pm 0.1	21.4 ^a \pm 0.3
ABTS	0.5 ^c \pm 0.1	7.9 ^b \pm 2.3	23.7 ^a \pm 0.2
DPPH	0.0 ^c \pm 0.0	2.5 ^b \pm 0.1	7.2 ^a \pm 0.5
Reducing power	0.2 ^c \pm 0.0	4.2 ^b \pm 0.1	7.9 ^a \pm 0.1
Superoxide anion	57.2 ^c \pm 0.6	76.0 ^b \pm 2.0	152.8 ^a \pm 3.3
BCB	20.1 ^b \pm 2.2	29.1 ^a \pm 2.7	33.8 ^a \pm 1.3
BCB (hexane)	40.6 ^c \pm 4.8	84.0 ^b \pm 1.6	108.9 ^a \pm 1.1

nd, not detected

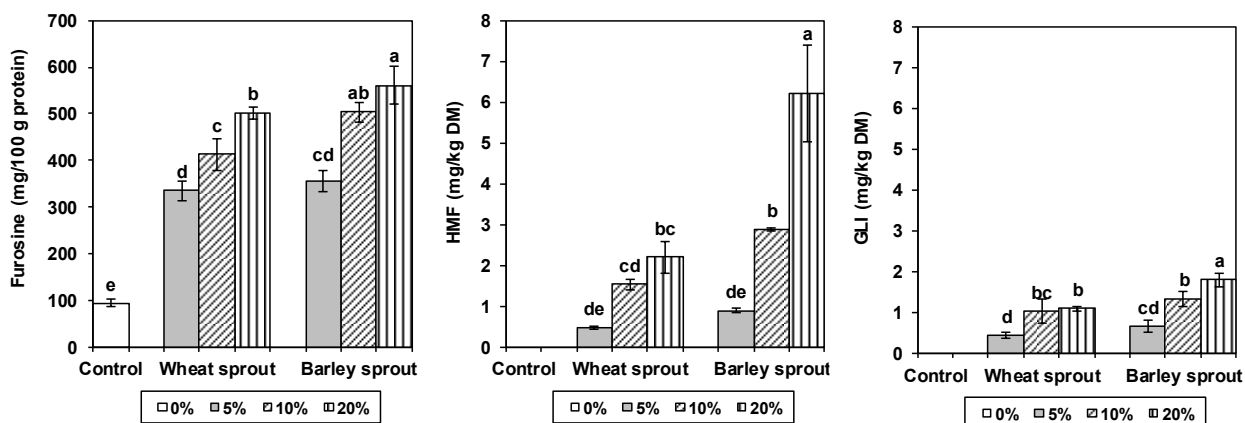


Figure 1

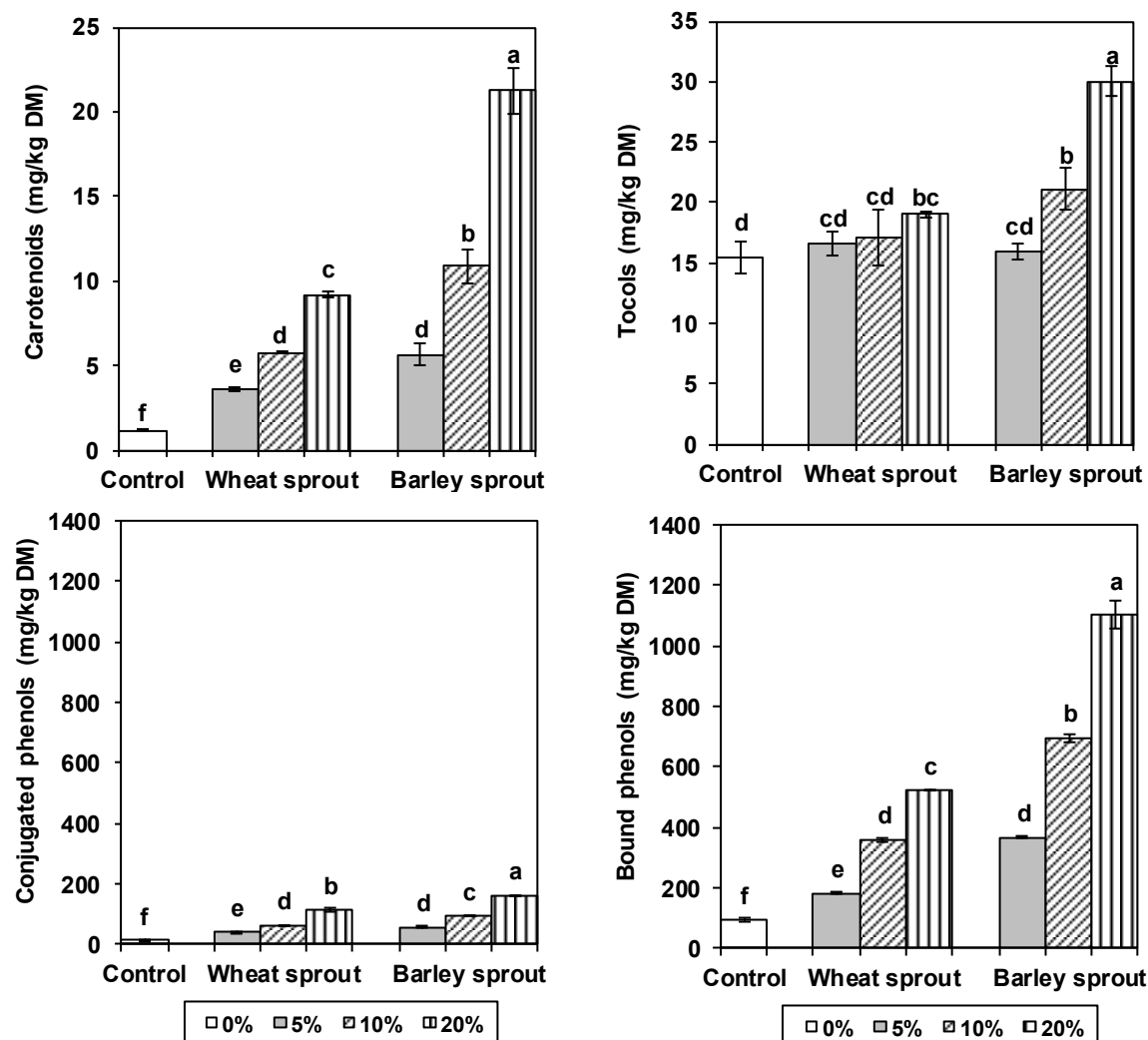
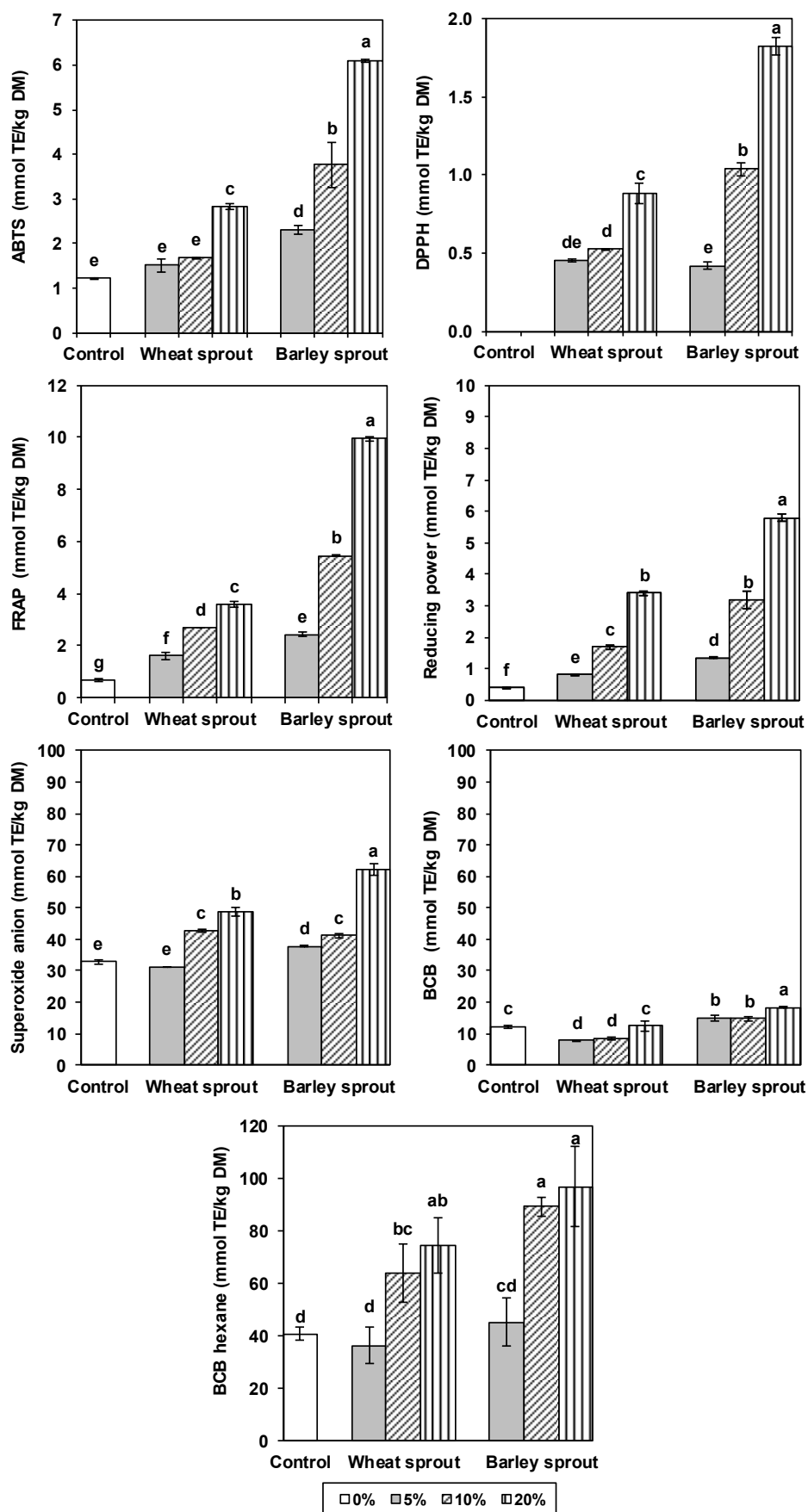


Figure 2



421

422 Figure 3

423 **Supplementary Table 1.** Mean (\pm standard deviation) and LSD test results ($p \leq 0.05$) for content in carotenoids, tocols,
424 soluble conjugated and insoluble bound phenolics (mg/kg DM) in biscuits obtained from bread wheat flour, without
425 (Control) and with addition (5%, 10% and 20%) of sprouted bread wheat meal (WS) or sprouted barley meal (BS).
426 Different letters indicate significant difference between the biscuits.

427

	Control	WS 5%	WS 10%	WS 20%	BS 5%	BS 10%	BS 20%
<i>Carotenoids</i>							
($\alpha + \beta$)-carotene	0.1 ^f \pm 0.0	0.7 ^e \pm 0.0	1.5 ^d \pm 0.1	2.8 ^c \pm 0.3	1.7 ^d \pm 0.1	3.8 ^b \pm 0.3	7.7 ^a \pm 0.5
β -cryptoxanthin	nd	nd	0.1 ^d \pm 0.0	0.1 ^c \pm 0.0	0.0 ^e \pm 0.0	0.2 ^b \pm 0.0	0.4 ^a \pm 0.0
Lutein	1.0 ^e \pm 0.1	2.7 ^d \pm 0.1	4.0 ^e \pm 0.0	5.9 ^b \pm 0.1	3.8 ^{cd} \pm 0.5	6.6 ^b \pm 0.8	12.6 ^a \pm 0.8
Zeaxanthin	0.1 ^d \pm 0.0	0.2 ^{bcd} \pm 0.0	0.2 ^{cd} \pm 0.0	0.4 ^b \pm 0.1	0.2 ^d \pm 0.0	0.3 ^{bc} \pm 0.0	0.5 ^a \pm 0.1
<i>Tocols</i>							
α -tocopherol	1.5 ^f \pm 0.1	2.1 ^{de} \pm 0.0	2.4 ^{cd} \pm 0.2	3.7 ^b \pm 0.5	1.7 ^{ef} \pm 0.1	2.8 ^c \pm 0.5	4.9 ^a \pm 0.1
α -tocotrienol	nd	0.6 ^d \pm 0.2	0.9 ^d \pm 0.1	1.3 ^c \pm 0.3	1.3 ^c \pm 0.0	3.1 ^b \pm 0.3	6.4 ^a \pm 0.0
β -tocopherol	2.7 \pm 0.4	2.8 \pm 0.2	2.7 \pm 0.5	2.8 \pm 0.2	2.4 \pm 0.2	2.7 \pm 0.3	3.5 \pm 0.5
β -tocotrienol	11.3 \pm 0.8	11.2 \pm 0.5	11.1 \pm 1.4	11.2 \pm 0.8	10.0 \pm 0.4	10.9 \pm 0.5	12.0 \pm 0.6
γ -tocopherol	nd	nd	nd	nd	0.6 ^c \pm 0.1	1.1 ^b \pm 0.2	2.2 ^a \pm 0.1
γ -tocotrienol	nd	nd	nd	nd	nd	0.4 ^b \pm 0.1	1.0 ^a \pm 0.0
<i>Conjugated phenolics</i>							
Chlorogenic	nd	nd	nd	nd	0.8 ^c \pm 0.3	1.5 ^b \pm 0.2	3.2 ^a \pm 0.1
Gentisic	0.0 ^e \pm 0.0	12.6 ^d \pm 2.4	19.1 ^d \pm 0.8	42.9 ^b \pm 6.6	19.4 ^d \pm 1.8	30.0 ^c \pm 5.6	55.5 ^a \pm 3.8
Vanillic	4.9 ^d \pm 0.0	7.3 ^c \pm 0.1	8.6 ^c \pm 0.2	11.9 ^{ab} \pm 0.2	7.8 ^c \pm 0.2	10.7 ^b \pm 0.4	13.3 ^a \pm 1.5
Syringic	1.0 ^f \pm 0.0	2.3 ^c \pm 0.1	3.8 ^b \pm 0.1	6.0 ^a \pm 0.0	1.5 ^e \pm 0.2	1.9 ^d \pm 0.0	2.2 ^c \pm 0.1
Coumaric	0.0 ^d \pm 0.0	3.4 ^c \pm 0.0	6.4 ^b \pm 0.4	10.9 ^a \pm 0.4	4.2 ^c \pm 0.4	6.8 ^b \pm 0.4	11.7 ^a \pm 0.7
Ferulic	4.4 ^f \pm 0.1	10.3 ^e \pm 0.4	18.2 ^c \pm 0.8	28.9 ^b \pm 0.9	14.3 ^d \pm 0.4	26.8 ^b \pm 1.3	45.2 ^a \pm 2.4
Salicylic	0.0 ^f \pm 0.0	2.2 ^c \pm 0.5	4.2 ^d \pm 0.3	7.6 ^c \pm 0.3	5.1 ^d \pm 0.0	10.8 ^b \pm 1	20.8 ^a \pm 0.6
Kaempferol	0.6 ^e \pm 0.0	1.3 ^{de} \pm 0.1	2.0 ^d \pm 0.3	3.3 ^c \pm 0.1	3.0 ^c \pm 0.0	5.0 ^b \pm 0.2	9.2 ^a \pm 0.9
<i>Bound phenolics</i>							
<i>p</i> -OHbenzoic	0.0 ^e \pm 0.0	5.1 ^d \pm 0.6	7.5 ^{bc} \pm 1.0	12.8 ^a \pm 0.2	6.6 ^{cd} \pm 0.1	8.8 ^b \pm 1.4	13.2 ^a \pm 0.2
Gentisic	0.0 ^d \pm 0.0	0.0 ^d \pm 0.0	2.6 ^c \pm 0.4	5.7 ^b \pm 0.7	3.0 ^c \pm 0.1	6.4 ^b \pm 0.6	9.7 ^a \pm 0.9
Vanillic	0.6 ^c \pm 0.1	0.9 ^c \pm 0.1	1.8 ^c \pm 0.2	2.4 ^b \pm 0.1	1.3 ^d \pm 0.1	2.4 ^b \pm 0.1	4.3 ^a \pm 0.3
Syringic	0.0 ^c \pm 0.0	0.0 ^c \pm 0.0	1.3 ^b \pm 0.2	1.8 ^a \pm 0	0.0 ^c \pm 0.0	0.0 ^c \pm 0.0	1.5 ^b \pm 0.2
Coumaric	2.3 ^c \pm 0.3	1.0 ^{de} \pm 0.0	3.3 ^d \pm 0.1	5.3 ^c \pm 0.0	6.0 ^c \pm 0.2	13.9 ^b \pm 1.0	22.7 ^a \pm 1.5
Ferulic	60.9 ^f \pm 2.9	117.8 ^e \pm 0.8	226.3 ^d \pm 7.1	320.5 ^c \pm 2.5	213.6 ^d \pm 6.5	397.4 ^b \pm 19.6	624.2 ^a \pm 0.5
Salicylic	28.2 ^e \pm 3.9	60.5 ^e \pm 1.9	122.3 ^d \pm 0.1	182.1 ^c \pm 1.1	138.7 ^d \pm 3.7	264.2 ^b \pm 7.3	423.6 ^a \pm 47.1
Kaempferol	0.0 ^c \pm 0.0	0.8 ^c \pm 0.1	1.8 ^d \pm 0.1	3.3 ^c \pm 0.4	3.1 ^c \pm 0.4	9.1 ^b \pm 0.6	15.9 ^a \pm 0.7

428 nd, not detected

429